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A novel mutation in the *TYRP1* gene associated with brown coat colour in the Australian Shepherd Dog Breed

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At least eight loci (A, B, D, E, H, K, M, S) are involved in coat colour variation in dogs. Locus B contains the *tyrosinase-related protein 1* (*TYRP1*) gene,[1] which encodes a melanosomal enzyme catalysing the oxidation of intermediates in the synthesis of eumelanin. *TYRP1* mutations cause dilution of the black eumelanin to brown. To date, three recessive mutations in *TYRP1* have been found in dogs with a brown coat colour: b^s, c.991C>T (p.Gln331Ter); b^d, c.1033_1035del (p.Pro345del); and b^c, c.121T>A (p.Ser41Cys).[1] The presence of two recessive alleles lead to the production of brown eumelanin. In a few breeds, such as the Australian Shepherd, the brown coat colour is confusingly called red. Here, we investigated the litter of an Australian Shepherd, in which two brown puppies appeared while the dam did not carry any of the three common *TYRP1* mutations.

Blood samples (K_3EDTA) or cytological brush samples were collected from 42 purebred dogs of the Australian Shepherd breed. Genomic DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen). PCR products generated by primers described previously[2] or newly designed for exon 7 (Table S1) were sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit and an ABI 3130xl Genetic Analyser (Applied Biosystems).

The presence of the mutation c.914C>T (p.Arg306Ter)[3] in the *melanocortin receptor* 1 (*MC1R*) gene was excluded in all investigated dogs. Therefore, the observed phenotypes were not caused by the production of pheomelanin instead of eumelanin. Sequencing of *TYRP1* revealed a novel nonsense mutation c.555T>G (p.Tyr185Ter, g.33319349T>G) (GenBank accession no. KY564174) in exon 2, resulting in a premature UAG stop codon and a truncation of 353 of the 537 amino acid residues, which contain all functional domains. Most likely, the brown coat colour in one sibling and two puppies of the dam is explained by the novel mutation in combination with the common mutation b^d or b^s (Fig. S1, Table S2). The *TYRP1*:c.555T>G mutation was present in four direct siblings of the dam and also in her mother but not in 30 non-related Australian Shepherd dogs. Nonsense mutations in exon 2 also have been reported in rabbits[4] and cats.[5,6]

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References

1 Schmutz S.M. *et al.* (2002) *Mamm Genome* **13**, 380–7.
2 Monteagudo L.V. & Tejedor M.T. (2015) *Anim Genet* **46**, 588–9.
3 Newton J.M. *et al.* (2000) *Mamm Genome* **11**, 24–30.
4 Utzeri V.J. *et al.* (2014) *Anim Genet* **45**, 600–3.
5 Lyons L.A. *et al.* (2005) *Anim Genet* **36**, 119–26.
6 Schmidt-Kuntzel A. *et al.* (2005) *J Hered* **96**, 289–301.

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Supporting information

Additional supporting information may be found online in the supporting information tab for this article:

Figure S1 Pedigree tree of the family illustrates the occurrence of a novel mutation.

Table S1 Primers used for the characterization of the canine *TYRP1* gene.

Table S2 The summary of variants found in *TYRP1* gene sequence of the proband family.

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Underdominant KCC3b R31I association with blood sodium concentration in domestic sheep suggests role in oligomer function

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Table 1 Adjusted mean blood sodium concentrations by *SLC12A6* (KCC3b) R31I genotype.

Genotype	*n*	Adjusted mean sodium conc. (mM)
II[a]	47	128.9
IR[b]	109	120.6
RR[a]	140	126.1

Tukey-Kramer pairwise comparisons: II[a] > IR[b] ($P = 0.008$); RR[a] > IR[b] ($P = 0.02$); RR[a] not different than II[a] ($P = 0.37$).

KCC3 and KCC1 are potassium chloride transporters encoded by *SLC12A6* and *SLC12A4* respectively with partially overlapping function,[1] and KCC3 knockout mice exhibit hypertension.[2] Two KCC3 isoforms differ by alternate promoters and first coding exons[3]: KCC3a is widely expressed and KCC3b is highly expressed in kidney proximal convoluted tubule.[4] We genotyped KCC3 and KCC1 amino acid substitutions using Taqman assays (Table S1) in 307 Suffolk, Rambouillet, Polypay and Columbia sheep. Whole blood sodium and potassium concentrations were determined by atomic absorbance spectrometry. Association was determined by mixed models in SAS 9.2 (SAS Institute, Cary, NC, USA) with breed and genotype for variant of interest as fixed effects and sire nested within breed treated as random. Preliminary testing showed age in years was not related to ion concentrations ($P > 0.05$), so age was not included in the models. No KCC3a or KCC1 substitutions (Table S2) were associated with blood potassium or sodium (all $P > 0.05$). KCC3b R31I is a charged substitution located in a conserved motif (Table S3), and although it was not associated with potassium ($P > 0.05$), it was associated with blood sodium in an underdominant manner (Table 1) whereby sodium was significantly higher in both homozygotes than in heterozygotes ($P < 0.05$). The sodium association is interesting because: (i) KCC3 is known to transport only potassium and chloride, not sodium[5] and (ii) KCC3a interacts with the sodium–potassium pump, whereas KCC3b does not.[6] Possible mechanisms include: (i) R31I alteration of KCC3b sodium affinity, (ii) KCC3b oligomer formation with sodium transporters[7] or (iii) indirect influence, e.g. by impairing sodium–potassium pump efficiency through potassium availability.[8] Regardless of the specific mechanism, the underdominant pattern suggests allelic incompatibility[9] such as dimer impairment. Because KCC3 functions as a homodimer,[10] R31I may interfere with KCC3b dimer function in regulating sodium concentration. To our knowledge, this is the first report of a KCC3 variant associated with blood sodium. These data suggest further study of coordinated function between KCC3 and sodium transport, and that sheep with KCC3b R31I may serve as a biomedical model.

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Conflict of interest

The authors declare no conflicts of interest exist.

References

1 Gagnon K.B. *et al.* (2013) *Am J Physiol Cell Physiol* **304**, C693–714.
2 Adragna N.C. *et al.* (2004) *Adv Exp Med Biol* **559**, 379–85.
3 Mercado A. *et al.* (2005) *Am J Physiol Renal Physiol* **289**, F1246–61.
4 Melo Z. *et al.* (2013) *Am J Physiol Renal Physiol* **305**, F1402–11.
5 Hiki K. *et al.* (1999) *J Biol Chem* **274**, 10661–7.
6 Fujii T. *et al.* (2010) *Biochem Biophys Res Commun* **399**, 683–7.
7 Simard C.F. *et al.* (2007) *J Biol Chem* **282**, 18083–93.
8 Garrahan P.J. *et al.* (1967) *J Physiol* **192**, 159–74.
9 Laiba E. *et al.* (2016) *Genome* **59**, 231–42.
10 Salin-Cantegrel A *et al.* (2011) *J Biol Chem* **286**, 28456–65.

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Supporting information

Additional supporting information may be found online in the supporting information tab for this article:

Table S1 Genotyping reagents.

Table S2 Breed allele frequencies.

Table S3 Amino acid alignment of mammal KCC3b highlighting R31I.